

THE ACTION OF CARBON MONOXIDE ON CERTAIN BLOOD PROTOZOA

BY

V. DOGIEL

AND

T. FEDOROWA

(LENINGRAD)

(Received for publication 25 May, 1926)

The general idea, which led us to begin the experiments described in this paper, was to elucidate the question, if carbon monoxide cannot be proved to exert a certain toxic influence on different blood-parasites (*Haemosporidia*, *Trypanosoma*, etc.). It is a well-known fact that the inhalation even of very small quantities of CO not only produces a poisonous effect on the higher terrestrial vertebrates (Mammalia, Aves), but at the same time deprives the erythrocytes of these animals of their capacity to absorb the oxygen contained in the lungs. Therefore it is to be presumed that the quantity of oxygen contained in the blood, and especially in the erythrocytes, of the vertebrates poisoned by CO, is considerably smaller than that of normal animals. If so, then it is permitted to surmise, *a priori*, that the change of oxygen-content in the erythrocytes may prove deleterious for blood-parasites included therein. If, on the other hand, the dose of CO fatal for the blood-parasites can be supported without serious damage by their host, then a new departure in the methods of struggle against blood-parasites will be opened. The main feature of the action on the parasites, proposed by us, consists in injuring their respiratory functions. The fact that *Plasmodium*, *Karyolysus*, *Babesia*, etc., choose the erythrocytes as their abode, seems to indicate that these Protozoa are in constant need of oxygen, so that a lack of this gas must influence their vitality. In this respect our experiments differ entirely from the procedure recently employed by Cleveland (1925) for disinfection of the gut-contents of termites, frogs, etc., and for the killing of their intestinal Protozoa. Cleveland, as appears from his very interesting papers, used, for his purposes, the oxidising action of oxygen, keeping the test-animals in sealed jars with pure oxygen at $3\frac{1}{2}$ atmospheres pressure.

Our work was begun quite independently of the American author, in the month of March, 1925, but owing to the lack of material, was protracted until the autumn of the same year.

In the vicinity of Leningrad there are very few animals harbouring blood-parasites, so that we were obliged to use common lizards (*Lacerta vivipara*) infected by the Haemogregarines of the genus *Karyolysus*. In addition, a few experiments were conducted with the rice-bird (*Padda oryzivora*) procured from abroad and infected by *Haemoproteus oryzivorae*. Even this material was not too abundant, because a great percentage of the lizards procured was free from parasites. During the whole summer we were able to obtain about 200 lizards, 110 being young, and the remainder adult. The young ones (of the brood of the same year) were never infected, whereas sixteen of the adult lizards were more or less heavily infected with *Karyolysus*. Of this number a certain number perished at the beginning of the experiments owing to insufficient care in handling them, so that the number of successfully conducted experiments became relatively small. But as we are by no means certain that the circumstances will permit us to continue our work during the summer of 1926, and as they appear to give some positive results, we take the liberty of publishing a preliminary note of our results.

The experiments were conducted thus:—The infected lizards were put under a glass bell-jar, which was fixed on a board by means of a rubber ring, and the edge of the jar was hermetically sealed by a thick varnish. The capacity of the bell-jar in different experiments varied from $1\frac{1}{2}$ to 10 litres, but it was always such that the animals could survive with no visible deleterious effects when the jar contained normal air. Through a hole in the board CO was introduced by means of some tubing. A thermometer, if needed, was placed under the jar at the beginning of the experiment.

In experiments with birds, ordinary room-temperature (16-17° C.) can be used, because the birds are highly susceptible to the action of CO. The lizards, on the contrary, can endure a very long sojourn in CO of high concentration (for instance, in air containing 7 per cent. of CO) without being poisoned by it. This circumstance obliged us to combine the action of CO with that of high temperature, which combination proved to be of greater effect. The animals were kept in the jar until marked symptoms of suffocation (convulsions,

acceleration of the respiratory movements, closing of the eyes, etc.) were observed. Then the jar was removed and the animal was allowed to recover (for five to fifteen minutes), when the experiment was renewed. The continued repetition of the experiment was prompted by the idea that for successful action on the blood-parasites, the whole amount of blood of the animal must come in contact with CO. The amount of time passed by the animals in the atmosphere of CO differed greatly in different experiments. Thus birds survive exposure to CO, even of low concentration only, three to fifteen minutes, while lizards survive for several hours.

To estimate the effect of the treatment with CO, blood smears were taken just before and after each experiment. In lizards the blood was taken by cutting off a small piece of the tail, in birds the blood was got from the leg. The cut was treated with iodine to prevent bacterial infection. The smears were stained by Giemsa, and the number of parasites present in a certain number of microscopic fields (Zeiss Ocular 7, Obj. Hom. Immersion 2 mm.) was carefully counted.

Lizards. The erythrocytes of *Lacerta vivipara* contain the merozoites of *Karyolysus* in the form of small worm-like bodies. The a-sexual reproduction (schizogony) of *Karyolysus* proceeds not in the blood, but in the internal organs (spleen). This circumstance evidently can affect the results of the experiments, as the number of the parasites in the blood can be increased at the expense of the spleen-parasites; but in cases where the number of parasites in the blood is, nevertheless, markedly reduced after the experiment, the experiment may be considered to be positive.

The first experiments with lizards showed that they could endure an atmosphere of CO for twenty-seven to forty-eight hours, without experiencing any visible discomfort. The action of CO being too slight, we decided to combine the action of the gas with that of a high temperature produced by direct sunlight. The jars were exposed to the direct influence of the sun's rays. The temperature rose to 38°-42°. The lizards invariably died after thirty to forty-five minutes. If taken out of the jar in time, they recovered, although sometimes it was necessary to have recourse to artificial respiration, and it was necessary to be sharply on the look-out for suspicious symptoms, otherwise the animal was past recovery. After several minutes of respite the experiment was resumed.

The following records were obtained :—

LIZARD NO. I.

	Number of micro. fields examined	Number of parasites found	Date	Remarks
1. Before the first experiment ...	200	133	22 July	—
2. After the first experiment ...	200	34	22 July	2 hours 30 minutes under the bell-jar (with short intervals) in 1 per cent. CO. About half of the time under direct sun-rays.
3. Before the second experiment...	200	13	24 July	—
4. After the second experiment ...	200	8	24 July	2 hours under the bell-jar (with short intervals).
5. After a long period of life in normal conditions	200	5	15 Aug.	—

Lizard No. I died at the end of August, of unknown causes, but to the end of its life it continued to show only a minimal number of parasites.

LIZARD NO. II.

	Number of micro. fields examined	Number of parasites found	Date	Remarks
1. Before the experiment ...	200	444	25 July	—
2. After the experiment	200	226	25 July	2 hours (with short intervals) in 1 per cent. CO. Evening, the sun not very hot.
3. After the second experiment ...	200	150	26 July	35 minutes in 1 per cent. CO. Sun very hot; the lizard died after the experiment, not withstanding the artificial respiration.

LIZARD No. III.

	Number of micro. fields examined	Number of parasites found	Date	Remarks
1. Before the experiment ...	300	272	23 Aug.	—
2. After the experiment	300	87	23 Aug.	45 minutes (with one interval) in 1 per cent. CO.

LIZARD No. IV.

	Number of micro. fields examined	Number of parasites found	Date	Remarks
1. Before the experiment ...	200	1,026	29 July	—
2. After the experiment	200	572	30 July	45 minutes in CO. of very low concentration (0.25 per cent.). Sun very hot; the animal was restored to life only by means of artificial respiration.
3. Before the second experiment...	200	666	10 Aug.	—
4. After the second experiment ...	200	372	10 Aug.	2 hours (with interval) in 2 per cent. CO. The animal died at the end of the second experiment.

The data show that in lizards I-IV the number of blood-parasites was greatly reduced by the treatment, although a total extermination of *Karyolysus* could not be attained. In the case of lizard No. I, the reduction of the number of parasites attained continued for a long period, so that the parasites in the internal organs seem to have been destroyed.

Experiments carried out by exposure to heat alone gave a negative result, and it appears to be the case that only the combined effect of the high temperature and CO was effective.

Birds. A specimen (*Padda oryzivora*) was exposed to 0.5-1 per cent. CO, at a temperature of 15-17° C., in a jar of 10 litres

capacity, for two-and-a-half to three hours (with several short intervals) during one experiment. Such experiments were repeated with one bird seven different times, till it died, but no reduction of the number of gametocytes could be observed. It is interesting to compare these results with the experiments of Cleveland, on the toxicity of oxygen to the intestinal Protozoa. Cleveland has, so far, likewise failed to affect the Protozoa of the higher vertebrates possessing a constant blood-temperature, while he succeeded in destroying the parasites of the frog.

Although our experiments are far from conclusive, however, the marked reduction in the numbers of parasites in the case of lizards treated by CO is evident and calls for further research.